REMARKS

Claim 9 has been amended to remove the phrase "substantially free of endogenous cells" and to add the phrase "graft composition is prepared by providing liver basement membrane substantially free of cells and seeding the hepatocytes on the liver basement membrane substantially free of cells." Support for amended claim 9 can be found in the specification, for example, on page 1, line 31 to page 2, line 2, and on page 2, line 30 to page 3, line 3, of the specification as filed. Additional minor amendments have been made to claim 9 to address clarity issues raised by the Examiner.

Rejection of Claims 9-16 Under 35 U.S.C. § 112, second paragraph-

The Examiner has rejected claims 9-16 under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner contends that claim 9 comprises two "administering" steps and is unclear. Further, the Examiner contends that the phrase "the liver basement membrane is seeded with hepatocytes for a time sufficient to allow said hepatocytes to grow," is unclear. Applicants have amended claim 9 to remove the phrase indicating that the "liver basement membrane is seeded with hepatocytes for a time sufficient to allow said hepatocytes to grow." In addition, Applicants have amended claim 9 to remove the phrase "administering to a patient." Accordingly, the Examiner's rejection under 35 U.S.C. § 112, second paragraph, has been rendered moot. Withdrawal of the rejection of amended claims 9-16 under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Rejection of Claims 9-16 Under 35 U.S.C. §103(a)-

The Examiner has rejected claims 9-16 under 35 U.S.C. §103 as being obvious over WO 98/25637 and U.S. Patent No. 6,793,939 each in view of U.S. Patent No. 5,510,254

(hereinafter "Naughton et al."). WO 98/25637 and U.S. Patent No. 6,793,939 are the PCT publication and an issued U.S. patent based on the PCT application from which the WO 98/25637 publication resulted, referred to hereinafter collectively as "Badylak."

The Examiner contends that it would have been within the knowledge of one skilled in the art to employ the co-culture technique of Naughton et al. in the method of Badylak to arrive at a method where hepatocytes and stromal cells may be co-cultured on liver basement membrane for administration to a patient in need of liver repair. The Examiner further contends that one would have had a reasonable expectation that the hepatocytes would retain their tissue-specific functions, including albumin production, urea production, and cytochrome P450 activity. Applicants respectfully traverse the Examiner's rejection. Claims 9-16 of the instant application are not obvious under 35 U.S.C. § 103(a).

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art. *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385, 1396 (2007). Although Applicants disagree that the Examiner has established a *prima facie* case of obviousness sufficient to support the Examiner's rejections under 35 U.S.C. §103, the Examiner's rejections under 35 U.S.C. § 103(a) are overcome for the following reasons.

A. REFERENCES MUST BE CONSIDERED AS A WHOLE, INCLUDING PORTIONS THAT WOULD LEAD AWAY FROM THE CLAIMED INVENTION -

The Examiner asserts that Naughton et al. establishes that means were known in the art, before the filing date of the instant application, for culturing hepatocytes while maintaining their functionality. However, Naughton et al. teaches away from amended claims 9-16 of the instant application. Ascertaining the differences between the prior art and the claims at issue requires interpreting the claim language, and considering both the

invention and the prior art references as a whole. A prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984) and MPEP § 2145.

Applicants have amended independent claim 9 to specify that the "graft composition is prepared by providing liver basement membrane substantially free of cells and seeding the hepatocytes on the liver basement membrane substantially free of cells." Naughton et al. teaches that *in vitro* culture of liver parenchymal cells requires growth on a pre-established stromal tissue comprising stromal cells (e.g., endothelial cells, adipocytes, fibroblastic cells, and Kupffer cells). Naughton et al. further teaches that the stromal tissue, including stromal cells, provides the support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of liver parenchymal cells in culture. *See* Naughton et al., col. 4, lines 50-59; col. 7, lines 4-9; col. 10, lines 26-28; and col. 10, lines 50-53. The only examples of hepatocyte functionality (e.g., P450 enzyme activity and albumin production) described in Naughton et al. include inoculation of liver parenchymal cells on pre-established stromal cells (*e.g., See* Naughton et al., Figure 5 and Figure 4A). In fact, the Examiner states that "Naughton et al. teach hepatocytes can be cultured in vitro while retaining their functionality *by co-culturing the hepatocytes with stromal cells*." See page 3 of the March 21, 2009 Office Action, emphasis added.

Thus, Badylak, as indicated by the Examiner, does not report on the functionality of hepatocytes cultured on liver basement membrane (See page 6 of the March 12, 2009 Office Action). Further, Naughton et al. teaches away from Applicants' claimed invention, i.e., the maintenance of *functional* hepatocytes in culture by seeding the hepatocytes on *liver basement membrane substantially free of cells*, because Naughton et al.

teaches that pre-established stromal cells are required for maintenance of parenchymal cell functionality in culture. Therefore, the reference combination does nothing to render amended claims 9-16 of the instant application obvious. Accordingly, withdrawal of the rejection of amended claims 9-16 under 35 U.S.C. §103(a) is respectfully requested.

B. GREATER THAN EXPECTED RESULTS ARE EVIDENCE OF NONOBVIOUSNESS-

Applicants have shown that *functional* hepatocytes can be maintained in culture by seeding the hepatocytes on *liver basement membrane substantially free of cells*. As the court concluded in *In re Diamond*, the question of nonobviousness must turn on whether the *prima facie* case of obviousness of the claimed composition is rebutted by a showing of unexpected results. *In re Diamond*, 53 CCPA 1172, 360 F.2d 214, 149 USPQ 562 (1966). *In re Meinhardt*, 55 CCPA 1000, 392 F.2d 273, 157 USPQ 270 (1968). A showing that the results obtained were greater than those which would have been expected from the prior art, and that the results are of a significant, practical advantage, is evidence of nonobviousness. *See* MPEP § 7.16(a); *Ex parte The NutraSweet Co.*, 19 USPQ2d 1586 (Bd. Pat. App. & Inter. 1991).

As indicated by the Examiner, Badylak mentions that liver basement membranes might be useful to *stimulate proliferation* of undifferentiated or differentiated cells (column 8, lines 55-59 and page 12, lines 3-6, respectively). Each Badylak reference also lists examples of differentiated cells, including hepatocytes. However, even if one skilled in the art expected to stimulate the *proliferation* of hepatocytes (*i.e.*, increase their number) by using liver basement membrane compositions as a substrate, there is no expectation that liver basement membranes could be used for *maintenance of hepatocyte functionality*, wherein the graft composition is prepared by seeding the hepatocytes on *liver*

basement membrane substantially free of cells. Mere hepatocyte proliferation in culture does not translate into maintenance of hepatocyte functionality.

Furthermore, Naughton et al. describes growth of liver parenchymal cells on stromal tissue, including pre-established stromal cells. Naughton et al. indicates that the stromal tissue, including pre-established stromal cells, is required to provide the support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of liver parenchymal cells in culture. *See* Naughton et al., col. 4, lines 50-59; col. 7, lines 4-9; col. 10, lines 26-28; and col. 10, lines 50-53. In fact, the only examples of hepatocyte functionality (e.g., P450 enzyme activity and albumin production) described in Naughton et al. include inoculation of liver parenchymal cells on pre-established stromal cells. Therefore, one skilled in the art would not expect to maintain *functional* hepatocytes by seeding the hepatocytes on *liver basement membrane substantially free of cells*, as specified in Applicants' amended claims 9-16.

Specifically, hepatocyte culture on liver basement membrane was found by Applicants to be unexpectedly superior to conventional hepatocyte culture on adsorbed collagen and hepatocytes were found to maintain synthetic and metabolic functions when cultured on liver basement membranes substantially free of cells. For example, albumin production, a measure of liver synthetic function, was found to be maintained or elevated in hepatocytes cultured on substantially cell free liver basement membrane, whereas albumin production declined in hepatocytes cultured on adsorbed collagen. (See page 22, lines 19-21 in the instant application). The synthetic and metabolic functions were not only superior to culture on adsorbed collagen, but were also comparable to culture on a double-gel collagen substrate, a substrate with known capacity for maintaining hepatocyte synthetic and metabolic functions. (See page 22, lines 16-18 in the instant application).

Also, urea content, a measure of liver metabolic function, was found to be about the same in hepatocytes cultured on liver basement membrane substantially free of cells, on a per cell basis, as that from cells grown on a double-gel substrate. (See page 22, line 32 to page 23, line 1 in the instant application). Additionally, in hepatocytes cultured on liver basement membrane substantially free of cells, cytochrome P450 activity, a measure of liver metabolic activity, was at least as high if not greater than that for hepatocytes grown on a double-gel substrate. (See page 23, lines 27-31 in the instant application). These results together show that hepatocytes grown on liver basement membranes substantially free of cells exhibit specific liver synthetic and metabolic activity characteristic of *functional hepatocytes*, results that are unexpected based on the prior art.

Accordingly, even if the Examiner has established a *prima facie* case of obviousness, and Applicants contend that the Examiner has not, Applicants have overcome the Examiner's *prima facie* case of obviousness by demonstrating that Applicants' claimed methods and compositions unexpectedly result in a level of hepatocyte functionality that is difficult to obtain. Furthermore, Naughton et al, as cited by the Examiner, teaches away from Applicants' claimed invention, and the cited reference combination does nothing to render amended claims 9-16 of the instant application obvious. Withdrawal of the rejection of amended claims 9-16 under 35 U.S.C. § 103(a) is respectfully requested.

Double Patenting -

(1) The Examiner has rejected claims 9-16 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-14 of U.S. Patent No. 6,793,939 in view of WO 98/25637, and further in view of U.S. Patent No. 5,510,254. Applicants have amended independent claim 9 to specify that the graft composition is

prepared by seeding hepatocytes on liver basement membrane substantially free of cells. Amended claims 9-16 are not obvious over claims 1-14 of U.S. Patent No. 6,793,939 in view of WO 98/25637, and further in view of U.S. Patent No. 5,510,254. The claims of U.S. Patent No. 6,793,939 provide no suggestion that *functional* hepatocytes can be maintained in culture by seeding the hepatocytes on *liver basement membrane substantially free of cells*. Furthermore, the secondary references cited by the Examiner do not provide what the claims of U.S. Patent No. 6,793,939 are missing (*See* arguments above for the rejection under 35 U.S.C. § 103). Accordingly, amended claims 9-16 cannot be obvious over claims 1-14 of U.S. Patent No. 6,793,939 in view of WO 98/25637, and further in view of U.S. Patent No. 5,510,254. Applicants respectfully request that the rejection of claims 9-16 on the basis of obviousness-type double patenting be withdrawn.

(2) The Examiner has rejected claims 9 and 12 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, 12, and 14 of U.S. Patent No. 7,482,025 in view of WO 98/25637, and further in view of U.S. Patent No. 5,510,254. Applicants have amended independent claim 9 to specify that the graft composition is prepared by seeding hepatocytes on liver basement membrane substantially free of cells. Amended claims 9-16 are not obvious over claims 1, 3, 12, and 14 of U.S. Patent No. 7,482,025 in view of WO 98/25637, and further in view of U.S. Patent No. 5,510,254. The claims of U.S. Patent No. 7,482,025 provide no suggestion that *functional* hepatocytes can be maintained in culture by seeding the hepatocytes on *liver basement membrane substantially free of cells*. Furthermore, the secondary references cited by the Examiner do not provide what the claims of U.S. Patent No. 7,482,025 are missing (*See* arguments above for the rejection under 35 U.S.C. § 103). Accordingly, amended claims 9-16 cannot be obvious over claims 1, 3, 12, and 14 of U.S. Patent No. 7,482,025 in view of

WO 98/25637, and further in view of U.S. Patent No. 5,510,254. Applicants respectfully request that the rejection of claims 9-16 on the basis of obviousness-type double patenting be withdrawn.

CONCLUSION

The foregoing amendments and remarks are believed to fully respond to the Examiner' s rejections. The amended claims are in condition for allowance. Applicants respectfully request allowance of the claims, and passage of the application to issuance.

Respectfully submitted,

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